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ARTICLE

Association of *MC1R* Variants and Host Phenotypes With Melanoma Risk in *CDKN2A* Mutation Carriers: A GenoMEL Study

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Background Carrying the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) germline mutations is associated with a high risk for melanoma. Penetrance of *CDKN2A* mutations is modified by pigmentation characteristics, nevus phenotypes, and some variants of the melanocortin-1 receptor gene (*MC1R*), which is known to have a role in the pigmentation process. However, investigation of the associations of both *MC1R* variants and host phenotypes with melanoma risk has been limited.

Methods We included 815 *CDKN2A* mutation carriers (473 affected, and 342 unaffected, with melanoma) from 186 families from 15 centers in Europe, North America, and Australia who participated in the Melanoma Genetics Consortium. In this family-based study, we assessed the associations of the four most frequent *MC1R* variants (V60L, V92M, R151C, and R160W) and the number of variants (1, ≥2 variants), alone or jointly with the host phenotypes (hair color, propensity to sunburn, and number of nevi), with melanoma risk in *CDKN2A* mutation carriers. These associations were estimated and tested using generalized estimating equations. All statistical tests were two-sided.

Results Carrying any one of the four most frequent *MC1R* variants (V60L, V92M, R151C, R160W) in *CDKN2A* mutation carriers was associated with a statistically significantly increased risk for melanoma across all continents ($1.24 \times 10^{-6} \leq P \leq .0007$). A consistent pattern of increase in melanoma risk was also associated with increase in number of *MC1R* variants. The risk of melanoma associated with at least two *MC1R* variants was 2.6-fold higher than the risk associated with only one variant (odds ratio = 5.83 [95% confidence interval = 3.60 to 9.46] vs 2.25 [95% confidence interval = 1.44 to 3.52]; $P_{trend} = 1.86 \times 10^{-8}$). The joint analysis of *MC1R* variants and host phenotypes showed statistically significant associations of melanoma risk, together with *MC1R* variants ($.0001 \leq P \leq .04$), hair color ($.006 \leq P \leq .06$), and number of nevi ($6.9 \times 10^{-6} \leq P \leq .02$).

Conclusion Results show that *MC1R* variants, hair color, and number of nevi were jointly associated with melanoma risk in *CDKN2A* mutation carriers. This joint association may have important consequences for risk assessments in familial settings.

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Cutaneous melanoma, the most common form of melanoma, is a complex disease that arises through multiple etiological pathways. The cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene (Mendelian Inheritance in Man 600160), located on chromosome 9p21, is known to be a major high–risk melanoma susceptibility gene that is transmitted according to a dominant mode of inheritance in melanoma-prone families (1,2). It encodes two distinct tumor suppressor proteins that are translated in alternative reading frames (ARFs) from alternate spliced transcripts (3–6). The alpha (α) transcript, comprises exons 1α, 2, and 3 and encodes the p16INK4a protein. This protein is known to inhibit the cyclin-dependent kinase 4 (CDK4)–mediated phosphorylation of retinoblastoma 1 protein and prevents the cell from progressing through the G_1 cell cycle checkpoint (3,4). The beta (β) transcript, comprises exons 1β, 2, and 3 and encodes the p14ARF protein. This protein acts via tumor protein p53 pathway to induce cell cycle

arrest or apoptosis (5,6). The germline *CDKN2A* mutations have been found in about 40% of melanoma-prone families from around the world (7). Most *CDKN2A* mutations are scattered through the lengths of exons 1α and 2, thus affecting p16INK4a protein alone or both p16INK4a and p14ARF proteins (7). The penetrance of *CDKN2A* mutations in multiple-case melanoma families was found to vary across continents, indicating that variations in genetic backgrounds, host characteristics, and/or sun exposure may contribute to the differences in penetrance (8).

Among the host phenotypes that may influence melanoma risk, dysplastic nevi, high numbers of banal nevi, poor tanning ability, and/or propensity to sunburn were shown to be associated with enhanced *CDKN2A* penetrance in melanoma-prone families (9,10). The melanocortin-1 receptor (*MC1R*) gene (Mendelian Inheritance in Man 155555), which plays a key role in the pigmentation process (11), has been consistently found to be a low-risk melanoma susceptibility gene in case–control studies, discussed in a review article (12). Moreover, *MC1R* variants have been shown to increase melanoma risk in families with *CDKN2A* mutations (13–16).

The *MC1R* gene is highly polymorphic in populations of European ancestry and more than 85 nonsynonymous variants have been identified (17,18). These include the red hair color (RHC) variants that are consistently associated with red hair, light skin, poor tanning ability, and heavy freckling, and the non-RHC (NRHC) variants that have a weaker, or no association, with red hair (19). In previous studies investigating the associations of *MC1R* variants with melanoma, whether conducted in case–control series or melanoma-prone families, the melanoma risk was mainly associated with the RHC variants, although it has also been reported to be influenced by certain NRHC variants (12,13–16), indicating that MC1R plays a role in melanoma development beyond that of pigmentation. Moreover, an increase in melanoma risk with an increase in number of *MC1R* variants has been reported in most (12,14–16), but not all (13,20), studies.

To date, there are limited investigations on the joint associations of *MC1R* variants, pigmentation, and nevus phenotypes, with melanoma risk in *CDKN2A* mutation carriers. Two studies (15,16) explored these joint associations (*MC1R* variants and pigmentation phenotypes, or *MC1R* variants and nevus phenotypes, or *MC1R* variants and pigmentation phenotypes and nevus phenotypes) but were conducted in relatively small number of *CDKN2A* mutation carriers. The joint associations of *MC1R* variants, pigmentation phenotypes, and/or nevus phenotypes with melanoma risk were tested in one study, but were restricted to RHC variants (16). Moreover, it is not known whether the associations of *MC1R* variants with melanoma risk in *CDKN2A* carriers vary, depending on whether the *CDKN2A* mutation alters p16INK4A protein alone or both p16INK4A and p14ARF proteins. Furthermore, *MC1R* variants have been inconsistently associated with a reduction in age at diagnosis of melanoma in *CKDN2A* mutation carriers (13–16).

The Melanoma Genetics Consortium (GenoMEL), which includes major familial melanoma research groups from Europe, North America, and Australia, has recruited the largest sample of *CDKN2A* mutation carriers, to our knowledge, to assess the association of *MC1R* variants with melanoma risk. In this study, we analyzed 815 *CDKN2A* mutation carriers that participated in GenoMEL to assess the association of *MC1R* variants, alone or in combination with host phenotypes, with melanoma risk in white populations with varying pigmentation characteristics and from countries and continents located at different latitudes with different patterns of sun exposure. We also explored whether the association of *MC1R* variants with melanoma risk differed if the *CDKN2A* mutations affected p16INK4a alone or both p16INK4 and p14ARF proteins.

CONTEXT AND CAVEATS

Prior knowledge

The association between cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutations and increased risk of cutaneous melanoma is influenced by host phenotypes (hair color, sunburn, number of nevi), as well as variants of melanocortin-1 receptor (*MC1R*), a gene associated with pigmentation characteristics and melanoma risk.

Study design

The association of *MC1R* variants, alone or jointly with host phenotypes, with melanoma risk was assessed in a family-based study of *CDKN2A* mutation carriers using GenoMEL participants from Europe, North America, and Australia.

Contribution

Each of the four frequent *MC1R* variants was associated with an increased melanoma risk in *CDKN2A* mutation carriers across all continents. The magnitude of the association of *MC1R* with melanoma increased consistently with an increase in the number of variants. Host phenotypes, analyzed individually or jointly, showed an association with increased melanoma risk. Both hair color and high numbers of nevi were associated with melanoma risk in addition to *MC1R* variants.

Implications

Increased melanoma risk in *CDKN2A* mutation carriers was associated jointly with host phenotypes and *MC1R* variants. These results are important for risk assessments in melanoma-prone families.

Limitations

The associations presented in this study are based on the sampled families and may not represent the general population.

From the Editors

Subjects and Methods

GenoMEL Subjects

Fifteen GenoMEL centers from Europe, North America, and Australia participated in the present analyses (Appendix). The following geographic locales were defined across three continents: Europe—France (Paris), Italy (Emilia-Romagna and Genoa), the Netherlands (Leiden), Spain (Barcelona), Sweden (Lund, Stockholm), and United Kingdom (Glasgow, Leeds); North America—Boston, NCI, Philadelphia, and Toronto; Australia—Brisbane and Sydney. Individual country locales were only considered within Europe in the analyses.

The *CDKN2A* mutation carriers from families with at least two cutaneous melanoma patients and presence of a germline *CDKN2A*

mutation in the family were eligible for the study. The types of *CDKN2A* mutations identified in the families included in this study are shown in Supplementary Table 1 (available online). Diagnoses of melanoma were confirmed by review of histology and pathology reports, medical records, or death certificates. To be eligible for the study, family members also had to have been genotyped for *MC1R*; approximately 94.2% of the *CDKN2A* mutation carriers were genotyped at the *MC1R* locus and were included in the analyses. For each continent, we checked whether the *CDKN2A* mutation carriers who were genotyped or not genotyped for *MC1R* differed in terms of melanoma affection status, sex, and age at examination and found that there was no difference (data not shown). Table 1 presents the total number of participants from 186 families (N = 815; 473 melanoma patients [affected], and 342 unaffected relatives with no manifestation of melanoma at the time the study was initiated) who were genotyped at the *CDKN2A* and *MC1R* loci by the GenoMEL study centers. The degree of familial relationship was estimated by examining all possible pairs of affected and unaffected subjects within families and their distribution was as follows—22% of affected and unaffected pairs were first-degree relatives, 23% were second-degree relatives, 20% were third-degree relatives, and the remaining 35% were more remote relatives. For all centers, written informed consent was obtained from all subjects before recruitment under an Institutional Review Board–approved protocol. Although the process of identifying and recruiting families differed among the GenoMEL centers because of variation in local health-care procedures and/or approaches for accruing families, the eligibility criteria for inclusion in this study were uniform, as described above. Details of the participating families and data collection have been described elsewhere (see Table 1 for references).

Genotyping of *CDKN2A* and *MC1R*

The protocol for detecting *CDKN2A* mutations has been described elsewhere; most families participating in the GenoMEL consortium were recently evaluated for the types of *CDKN2A* mutations and associated clinical factors, including age at melanoma diagnosis and presence of multiple primary melanomas in the family (7,30,31). The distribution of the *CDKN2A* mutations identified in the 186 families included in this study and their effect on p16INK4a and p14ARF proteins are shown in Supplementary Table 1 (available online).

The *MC1R* genotyping was performed in each center by sequencing the entire open reading frame of the single-exon gene in both affected patients and unaffected subjects. The methods varied slightly between the centers, and examples of methods can been found in Vajdic et al. (32) and Kanetsky et al. (33). We checked that the distribution of *MC1R* variants were in Hardy–Weinberg equilibrium; no departure from Hardy–Weinberg equilibrium at the 5% significance level was found. The Mendelian inconsistencies of the *MC1R* genotypes were checked using the PedCheck program (34), and any inconsistent genotypes were coded as missing data.

Table 1. Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutation carriers from the Melanoma Genetics Consortium (GenoMEL) and melanoma affection status in the *CDKN2A* mutation carriers genotyped for melanocortin-1 receptor (*MC1R*)* gene

Consortium participant			*CDKN2A* mutation carriers genotyped for *MC1R*		
Consortium center	Geographic locale, continent	No. of families; (reference)	No. of *CDKN2A* mutation carriers	No. of melanoma patients†	No. of unaffected subjects‡
Europe					
Paris	France, Europe	27 (16)	95	52	43
Emilia-Romagna	Italy, Europe	4 (21)	8	6	2
Genoa	Italy, Europe	14 (22)	38	26	12
Leiden	the Netherlands, Europe	8 (14)	113	52	61
Barcelona	Spain, Europe	16 (23)	66	30	36
Lund	Sweden, Europe	9	33	17	16
Stockholm	Sweden, Europe	4 (24)	15	5	10
Glasgow	United Kingdom, Europe	13 (25)	27	21	6
Leeds	United Kingdom, Europe	34 (26)	102	61	41
Subtotal		129	497	270	227
North America					
Boston	USA, North America	6 (27)	15	11	4
NCI	USA, North America	16 (15)	136	70	66
Philadelphia	USA, North America	2	10	9	1
Toronto	Canada, North America	15	32	26	6
Subtotal		39	193	116	77
Australia					
Brisbane	Australia	17 (28)	96	76	20
Sydney	Australia	1 (29)	29	11	18
Subtotal		18	125	87	38
Total		186	815	473	342

* The protocol for detecting *CDKN2A* mutations has been described elsewhere (7,30,31). *MC1R* genotyping was performed by sequencing the entire open reading frame of the single-exon gene (32,33). NCI = National Cancer Institute.

† The median ages of melanoma patients at examination were 47 years in Europe, 42 years in North America, and 54 years in Australia; and the median ages at diagnosis were 36 years in Europe, 31 years in North America, and 34 years in Australia.

‡ The median ages of unaffected subjects at examination were 41 years in Europe, 34 years in North America, and 47 years in Australia.

Data Collection

Information on familial relationships among members of the same family; demographic characteristics (sex; date of birth; and age at death and cause of death, if deceased); melanoma status (affected vs unaffected) together with confirmation of melanoma diagnosis and age at diagnosis; *CDKN2A* mutation status (no mutation [homozygote wild type] vs presence of a mutation [heterozygote or homozygote for the mutation]) and, in mutation carriers, location of the mutation in *CDKN2A* locus (promoter region, exons 1α, 1β, 2 and 3, and introns); *CDKN2A* nucleotide change and subsequent change in amino acid in p16INK4a and p14ARF proteins; *MC1R* genotypes (wild-type homozygotes for the consensus sequence, heterozygotes or homozygotes for a variant at each position of the sequence where an *MC1R* variant had been detected across all participating GenoMEL centers), pigmentation characteristics, and nevus phenotypes (including hair color, propensity to sunburn, nevus count) were obtained from each center using a standardized format based on a uniform coding scheme across GenoMEL centers. The markers of pigmentation and nevus phenotypes used in this study were coded as follows—hair color (classified as red, blond, brown, black); skin reaction to sun exposure (never burns, sometimes burns, usually burns, always burns); and nevus count (none, few, some and many nevi). The data received from each center were integrated into a common dataset using the Statistical Analysis System (SAS) software (version 9.1, developed by SAS Institute Inc, Cary, NC).

Statistical Analysis

Associations between *MC1R* variants and melanoma affection status (affected vs unaffected) were evaluated in *CDKN2A* mutation carriers using the generalized estimating equations (GEE) method to take into account familial dependences. The GEE method, a semiparametric regression method, specifies the relationship between the disease outcome (melanoma affection status) and predictor variables (eg, *MC1R* variants, host phenotypes) through a link function and takes into account the correlations among disease outcomes of family members through a correlation matrix. We used the logit link function and exchangeable correlation matrix, which assumes equal correlations among the disease outcomes in family members, to estimate odds ratios (ORs) and 95% confidence intervals (CIs). All analyses were adjusted for sex and age at examination as a continuous variable. We used age at examination for all subjects rather than age at diagnosis for melanoma patients because some host-related phenotypes like number of nevi vary with age and were assessed at time of examination. Tests of association between melanoma and predictor variables (*MC1R* variables as defined below and/or host phenotypes) were based on two-sided generalized score statistics.

Before conducting the association analysis of melanoma with *MC1R* variants, we estimated the frequency of each *MC1R* variant from individual genotypic data in *CDKN2A* mutation carriers (affected melanoma patients, unaffected subjects, and all subjects) from each continent, as well as all three continents combined. The frequent variants were those that had an estimated frequency greater than or equal to 5% in all *CDKN2A* mutation carriers from at least one continent and in all three continents (Table 2; Supplementary Table 2, available online). These frequent variants were the only ones that were analyzed individually.

We first investigated associations between melanoma and each frequent nonsynonymous *MC1R* variant individually by comparing carriage of at least one variant (homozygotes and heterozygotes pooled) to homozygosity for the *MC1R* consensus sequence (reference category). Because many *MC1R* variants were too rare to examine their individual association with melanoma risk in *CDKN2A* carriers, all nonsynonymous variants were grouped in various ways to make the following comparisons—carriers of any *MC1R* variant compared with homozygosity for the *MC1R* consensus sequence; carriers of multiple *MC1R* variants (1, ≥2 variants) compared with homozygosity for the *MC1R* consensus sequence; and carriers of specific types of *MC1R* variants (1 NRHC variant, 1 RHC variant, ≥2 NRHC variants, ≥2 RHC variants, or carriers of both RHC and NRHC variants) compared with *MC1R* consensus sequence. The RHC variants included four *MC1R* variants (R151C, R160W, D294H, and D84E), which have been consistently reported to be associated with RHC and light skin color; all other nonsynonymous variants were coded as NRHC.

Generalized score tests for association of melanoma risk with carriage of any *MC1R* variant and number of variants were conducted for each geographic locale separately, whereas tests for other *MC1R* variables (individual variants and types of variants) were conducted by continent (ie, pooling locales within Europe) because of small sample size in individual European countries. Pooled analyses of all locales within Europe and across continents were carried out by introducing a locale indicator variable in the regression model between disease outcome and predictor variables. This locale adjustment in the regression model can correct for potential population stratification because the locale variable was country specific for European mutation carriers in all analyses, whereas it was continent specific for North American and Australian mutation carriers because they were all of European ancestry and more than 70% were recruited from a single center, respectively (15,28). The homogeneity in the association of melanoma with any of the *MC1R* variables analyzed among geographic locales (as defined in the GenoMEL subjects paragraph) was tested by introducing *MC1R* variable × locale interaction terms in the regression model between melanoma and the *MC1R* variable, which also included sex, age at examination, and the locale indicator variable. These interaction terms, which are equal to zero under the null hypothesis of homogeneity of the association of *MC1R* variable with melanoma across geographic locales, were tested using a generalized score test which follows a χ^2 distribution with number of degrees of freedom equal to number of locales minus 1 for a given *MC1R* variable category. It must be noted that, for *MC1R* variables with only one category (eg, presence of a given *MC1R* variant or presence of any *MC1R* variant), there was only one interaction term per geographic locale, whereas for *MC1R* variables with more than one category (eg, number of *MC1R* variants and types of variants), there was one interaction term between each *MC1R* variable category and each geographic locale. We also used the Cochran *Q* test (35) to test for homogeneity of the estimates of the odd -ratios associated with each of the *MC1R* variables among European, North American, and Australian mutation carriers. We also investigated whether the associations of the frequent *MC1R* variants and number of *MC1R* variants with melanoma risk

Table 2. Frequency of melanocortin-1 receptor (*MC1R*) variants in cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutation carriers analyzed by continent and in all three continents*

		Europe		North America		Australia		All continents	
		No. of chromosomes† (n = 994)		No. of chromosomes† (n = 386)		No. of chromosomes† (n = 250)		No. of chromosomes† (n =1630)	
MC1R nucleotide change	MC1R amino acid change	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§
Consensus sequence‡	None	386	38.8	145	37.6	96	38.4	627	38.5
Nonsynonymous frequent variants‖									
g.178G>T	V60L	123	12.4	63	16.3	26	10.4	212	13.0
g.274G>A	V92M	105	10.5	29	7.5	21	8.4	155	9.5
g.451C>T	R151C	116	11.7	40	10.4	61	24.4	217	13.3
g.478C>T	R160W	94	9.5	33	8.5	17	6.8	144	8.8
Nonsynonymous rare variants¶									
g.44A>G	N15S	0	0	0	0	1	0.4	1	0.1
g.206A>C	H69P	1	0.1	0	0	0	0	1	0.1
g.247T>C	S83P	0	0	4	1.0	0	0	4	0.3
g.248C>T	S83L	2	0.2	0	0	0	0	2	0.1
g.252C>A	D84E	9	0.9	6	1.6	2	0.8	17	1.0
g.284C>T	T95M	3	0.3	0	0	0	0	3	0.2
g.364G>A	V122M	2	0.2	0	0	0	0	2	0.1
g.383T>A	M128K	1	0.1	0	0	0	0	1	0.1
g.425G>A	R142H	5	0.5	1	0.3	0	0	6	0.4
g.456C>A	Y152X	2	0.2	0	0	1	0.4	3	0.2
g.464T>C	I155T	12	1.2	10	2.6	1	0.4	23	1.4
g.488G>A	R163Q	51	5.1	12	3.1	11	4.4	74	4.5
g.512C>G	A171G	2	0.2	0	0	0	0	2	0.1
g.586T>C	F196L	1	0.1	0	0	0	0	1	0.1
g.652G>A	A218T	1	0.1	0	0	0	0	1	0.1
g.653C>G	A218G	1	0.1	0	0	0	0	1	0.1
g.662T>C	I221T	2	0.2	0	0	0	0	2	0.1
g.835A>G	N279D	5	0.5	0	0	0	0	5	0.3
g.880G>C	D294H	21	2.1	16	4.2	8	3.2	45	2.8
Insertions									
g_86_87insA		1	0.1	5	1.3	0	0	6	0.4
g_537_538insC		1	0.1	0	0	0	0	1	0.1
Synonymous frequent variants‖									
g.942A>G	T314T	102	10.3	41	10.7	9	3.7	152	9.3
Synonymous rare variants¶									
g.102G>C	R34R	0	0	1	0.3	0	0	1	0.1
g.399C>T	C133C	1	0.1	0	0	0	0	1	0.1
g.637C>A	R213R	1	0.1	0	0	0	0	1	0.1
g.699G>A	Q233Q	2	0.2	0	0	0	0	2	0.1
g.720T>C	A240A	0	0	1	0.3	0	0	1	0.1

(Table continues)

differed according to the type of *CDKN2A* mutations (mutations affecting p16INK4a protein only vs mutations affecting both p16INK4a and p14ARF proteins). The homogeneity of the odds ratios associated with these *MC1R* variables by type of *CDKN2A* mutations was tested by the Cochran *Q* test.

To investigate whether *MC1R* variants and host phenotypes were jointly associated with melanoma risk, we first assessed the association of each host phenotype alone with melanoma risk and then jointly examined the associations of *MC1R* variants and each phenotype and the associations of *MC1R* variants and all phenotypes with melanoma risk. These analyses were based on the GEE method, as described above. We considered various regression models between the melanoma affection status and predictor variables depending on the predictors included in the model: each host phenotype or all host phenotypes, with and without *MC1R* variants, while always adjusting for sex, age at examination, and geographic locale in the model. Regarding *MC1R*, we included in the regression model each frequent variant individually or the number of *MC1R* variants. The host phenotypes were dichotomized as follows—hair color (blond or red vs dark or brown), propensity to sunburn (usually or always burns vs sometimes or never burns) and nevus count (some or many nevi vs none or few nevi). Regarding hair color, the number of subjects with red hair (total, 57 subjects; affected, 47 subjects; unaffected, 10 subjects) precluded evaluation of red hair alone. Tests of associations between melanoma and each variable (host phenotypes and/or *MC1R* variants) were based on generalized score tests, as described before.

We also investigated whether the age at diagnosis of melanoma was influenced by *MC1R* variants. We estimated the median ages at melanoma diagnosis for each category of *MC1R* variables in melanoma patients from each continent and from all three continents. The nonparametric Jonckheere–Terpstra test was used to test the null hypothesis of no difference in the ages at diagnosis of melanoma across different categories of *MC1R* variables, against the alternative hypothesis of a change in age at diagnosis with the presence of any variant, presence of individual frequent variants, or as the number or number and types of *MC1R* variants increased.

All analyses were carried out using the SAS software (version 9.1). All statistical tests were two-sided.

Results

GenoMEL Participants Included in the Study and *MC1R* Variants

A total of 815 *CDKN2A* mutation carriers genotyped for *MC1R* were available for this study (Table 1). The percentage of melanoma patients (affected) was 54.3% in Europe, 60.1% in North America, and 69.6% in Australia. The median ages at examination among affected and unaffected *CDKN2A* mutation carriers were 47 and 41 years, respectively, in Europe; 42 and 34 years, respectively, in North America; and 54 and 47 years, respectively, in Australia. Among the melanoma patients, there were 43% men in Europe, 51% men in North America, and 54% men in Australia, whereas the percentages of men were 48%, 35%, and 47%, respectively, in unaffected subjects. No statistically significant difference in these proportions of men among affected melanoma patients or unaffected subjects was noted across continents ($P > .10$). No

Table 2 (continued).

		Europe		North America		Australia		All continents	
		No. of chromosomes† (n = 994)		No. of chromosomes† (n = 386)		No. of chromosomes† (n = 250)		No. of chromosomes† (n =1630)	
MC1R nucleotide change	MC1R amino acid change	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§	No. of chromosomes with and without *MC1R* variant‡	% of chromosomes with and without *MC1R* variant§
g.792C>T	I264I	0	0	3	0.8	0	0	3	0.2
g.948C>T	S316S	1	0.1	0	0	1	0.4	2	0.1

* The frequency of *MC1R* variants was estimated in all *CDKN2A* mutation carriers genotyped for *MC1R*. (*N* = 815: 497 from Europe, 193 from North America, and 125 from Australia).

† The number of chromosomes was twice the number of *CDKN2A* mutation carriers genotyped for *MC1R*.

‡ The number of chromosomes carrying no *MC1R* variant (consensus sequence) and those carrying a given variant are shown for all *CDKN2A* mutation carriers per continent and in all three continents.

§ The proportions shown in this table are the number of chromosomes carrying no *MC1R* variant (consensus sequence) or a given variant divided by the total number of chromosomes in *CDKN2A* mutation carriers per continent and in all three continents.

‖ The frequent variants shown in this table are those with an estimated frequency greater than or equal to 5% in *CDKN2A* mutation carriers from at least one continent and all three continents.

¶ The rare variants shown in this table are those with an estimated frequency less than 5% in *CDKN2A* mutation carriers from all three continents.

statistically significant difference in the median age at diagnosis of melanoma: 36 years in Europe, 31 years in North America, and 34 years in Australia ($P = .08$) was observed.

We estimated the frequency of each *MC1R* variant in *CDKN2A* mutation carriers from each continent and from all three continents (Table 2; details provided in Supplementary Table 2, available online). A total of 33 variants of *MC1R* were detected; 23 variants corresponded to nonsynonymous amino acid changes, eight variants corresponded to synonymous amino acid changes, and two variants corresponded to insertions. All subsequent analyses were restricted to nonsynonymous variants. Four nonsynonymous variants (V60L, V92M, R151C, and R160W) were observed at a frequency greater than or equal to 5% in at least one continent and all three continents in all mutation carriers (Table 2) as well as in affected and unaffected carriers (Supplementary Table 2, available online). The frequency of these variants did not differ statistically significantly across continents in unaffected mutation carriers ($P > .20$).

Associations of *MC1R* Variants With Melanoma Risk in *CDKN2A* Mutation Carriers

We first assessed the association of each of the four most frequent *MC1R* variants with melanoma risk in *CDKN2A* carriers. Both RHC (R151C and R160W) and NRHC (V60L and V92M) variants were associated with increased melanoma risk, but with varying strengths in different continents (Table 3). In Europe, this association was statistically significant for each variant, and the strongest association was noted for RHC variants, followed by NRHC variants ($.0002 \leq P \leq .03$). In North America, the association with increased melanoma risk reached statistical significance with R151C, R160W, and V92M variants ($.05 \leq P \leq .02$), whereas in Australia, the association reached statistical significance with V60L ($P = .009$) and R151C ($P = .03$) variants. The association of any of these *MC1R* variants with melanoma risk did not show evidence of heterogeneity across continents ($P_{homogeneity} \geq .09$). The pooled estimates of the odds ratios adjusted for age, sex, and locale were always higher than 2.0 and were lowest for V92M (OR = 2.43, 95% CI = 1.45 to 4.06) and highest for R151C (OR = 4.68, 95% CI = 2.52 to 8.68).

We carried out subsequent analyses by pooling all nonsynonymous variants in different ways—pooling all of them in one category (presence of any variant), pooling them according to their number (1, ≥2 variants), and pooling them according to their number and types (number of RHC and NRHC variants). When all nonsynonymous *MC1R* variants were pooled (Table 3), carrying at least one variant was associated with increased melanoma risk in all three continents ($.0006 \leq P \leq .05$). Some variation in the increase in melanoma risk was observed within Europe (Supplementary Table 3, available online). The highest increases in risk were observed in France, Spain, and Sweden ($.02 \leq P \leq .04$), whereas the odds ratio was close to unity in Italy, the country with the smallest sample size (Supplementary Table 3, available online). Nevertheless, tests for heterogeneity in the association of at least one *MC1R* variant with melanoma risk among geographic locales were not statistically significant ($P = .11$ within Europe; and $P = .16$ across all continents). In *CDKN2A* mutation carriers from all three continents, the presence of at least one *MC1R* variant was associated with a threefold increase in melanoma risk (OR = 3.05, 95% CI = 1.99 to 4.67). We then investigated whether there was an increase in melanoma risk associated with an increase in the number of *MC1R* variants. The increase in the number of *MC1R* variants showed a consistent increase in melanoma risk in *CDKN2A* mutation carriers from Europe, North America, and Australia (for Europe, $P_{trend} = 6.25 \times 10^{-6}$; for North America, $P_{trend} = .01$; and for Australia, $P_{trend} = .03$) (Table 3). This increase in risk with the increase in numbers of *MC1R* variants was seen in all European countries except Italy, where there was no increase in melanoma risk associated with either one *MC1R* variant or at least two variants (Supplementary Table 3, available online), and in Sweden, where the increase in melanoma risk associated with one variant was similar to the increase in risk associated with at least two variants (Supplementary Table 3, available online). No statistically significant heterogeneity for the increase in melanoma risk with the increase in number of *MC1R* variants was detected either across European countries ($P_{homogeneity} = .14$) or across all continents ($P_{homogeneity} = .23$). Overall, the risk associated with at least two *MC1R* variants was 2.6-fold higher than the risk associated with only one variant (OR = 5.83 [95% CI = 3.60 to 9.46] vs 2.25 [95% CI = 1.44 to 3.52]; $P_{trend} = 1.86 \times 10^{-8}$). Next, we explored whether there was an increase in melanoma risk with both the number and types of *MC1R* variants (RHC variants and NRHC variants). We observed that in all continents an increase in melanoma risk was associated with an increase in number of variants and, mostly, when the genotype included RHC variants (Table 3). The association with number and types of *MC1R* variants was homogeneous among continents ($P_{homogeneity} = .07$). The odds ratios estimated from all continents showed that there was a more than fivefold increase in melanoma risk in *CDKN2A* mutation carriers with at least two RHC variants compared with one NRHC variant (OR = 11.78 [95% CI = 5.34 to 26.02] vs 2.08 [95% CI = 1.28 to 3.40], respectively).

Because there was no statistically significant evidence for heterogeneity in the association of any of the studied *MC1R* variables with the melanoma risk across all geographic locales, we investigated whether the association of melanoma risk with individual *MC1R* variants and number of variants differed by the type of *CDKN2A* mutation in all *CDKN2A* mutation carriers from all three continents, while adjusting for age, sex, and geographic locale (Table 4). Overall, the point estimates of the odds ratios associated with each *MC1R* variant, except R160W, were higher when the *CDKN2A* mutations affected the p16INK4a protein alone, than when they affected both p16INK4a and p14ARF proteins. However, the confidence intervals were wide and tests of homogeneity for any *MC1R* variant according to the type of *CDKN2A* mutation were not significant ($P \geq .09$). Similar results were obtained when the analysis was done with the number of *MC1R* variants (Table 4).

Joint Associations of *MC1R* Variants and Host Phenotypes With Melanoma Risk in *CDKN2A* Mutation Carriers

Next we assessed the joint associations of *MC1R* variants and host phenotypes with melanoma risk in all *CDKN2A* mutation carriers from all three continents. We confirmed that, in the unaffected subjects, R151C and R160W variants were statistically significantly

Table 3. Association of individual melanocortin-1 receptor (*MC1R*) variants, number of *MC1R* variants, and types of *MC1R* variants with melanoma risk in cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutation carriers analyzed by continent and in all three continents*

	Europe			North America			Australia			All continents			
***MC1R* variants**	**No. of participants affected/ unaffected†**	**OR (95% CI)‡**	***P*§**	**No. of participants, affected/ unaffected†**	**OR (95% CI)‡**	***P*§**	**No. of participants, affected/ unaffected†**	**OR (95% CI)‡**	***P*§**	**No. of participants, affected/ unaffected†**	**OR (95% CI)‡**	***P*§**	$P_{homogeneity}$‖
Individual *MC1R* variants													
V60L	109/120	2.50 (1.40 to 4.46)	.003	50/41	4.45 (1.36 to 14.56)	.09	32/13	6.91 (2.10 to 22.75)	.009	191/174	3.42 (2.10 to 5.58)	1.51×10^{-5}	.38
V92M	91/117	2.03 (1.10 to 3.74)	.03	28/35	4.03 (1.08 to 14.98)	.03	24/17	2.51 (.85 to 7.41)	.12	143/169	2.43 (1.45 to 4.06)	.0007	.25
R151C	115/104	4.31 (1.81 to 10.23)	.0002	36/37	6.23 (2.25 to 17.28)	.02	57/20	5.58 (1.56 to 20.0)	.03	208/161	4.68 (2.52 to 8.68)	1.24×10^{-6}	.73
R160W	94/105	3.32 (1.77 to 6.21)	.001	35/32	15.04 (3.20 to 70.67)	.05	23/14	2.52 (.97 to 6.57)	.13	152/151	4.13 (2.30 to 7.43)	9.42×10^{-5}	.09
Any *MC1R* variant	270/227	2.59 (1.57 to 4.28)	.0006	116/77	5.67 (2.1 to 15.29)	.05	87/38	4.04 (1.53 to 10.65)	.02	472/342	3.05 (1.99 to 4.67)	2.39×10^{-5}	.16
No. of *MC1R* variants	270/227			116/77			87/38			473/342			.23
1		1.85 (1.09 to 3.15)	.02		3.93 (1.36 to 11.32)	.06		3.73 (1.67 to 8.30)	.02		2.25 (1.44 to 3.52)	.0009	
≥2		4.40 (2.56 to 7.57)	1.32×10^{-5}		13.57 (4.94 to 37.29)	.02		5.09 (1.25 to 20.74)	.03		5.83 (3.60 to 9.46)	9.66×10^{-8}	
P_{trend}¶			6.25×10^{-6}			.01			.03			1.86×10^{-8}	
Types of *MC1R* variants	270/227			116/77			87/38			473/342			.07
1 NRHC#		1.75 (.99 to 3.10)	.05		3.54 (1.22 to 10.25)	.04		2.55 (.66 to 9.84)	.16		2.08 (1.28 to 3.40)	.003	
1 RHC**		2.04 (1.08 to 3.85)	.03		4.36 (1.21 to 15.75)	.09		4.74 (1.41 to 15.99)	.02		2.59 (1.47 to 4.57)	.002	
≥ 2 NRHC		2.74 (1.31 to 5.77)	.01		8.00 (2.33 to 27.41)	.03		1.86 (.55 to 6.28)	.33		3.62 (1.90 to 6.89)	.0002	
1 RHC, 1 NRHC		4.43 (2.31 to 8.49)	4.9×10^{-5}		11.13 (3.44 to 35.97)	.01		6.12 (1.03 to 36.28)	.04		6.24 (3.43 to 11.34)	9.97×10^{-8}	
≥2 RHC		7.73 (3.61 to 16.58)	7.6×10^{-5}					7.72 (1.30 to 45.75)	.03		11.78 (5.34 to 26.02)	2.89×10^{-7}	

* The association of each *MC1R* variable (individual *MC1R* variants, any *MC1R* variant, number of *MC1R* variants, types of *MC1R* variants) with melanoma risk was estimated by using homozygosity for the *MC1R* consensus sequence as the reference category. CI = confidence interval; OR = odds ratio; NRHC = nonred hair color; RHC = red hair color.

† The number of GenoMEL participants contributing to the analysis of a given *MC1R* variable (individual *MC1R* variants, any *MC1R* variant, number of *MC1R* variants, types of *MC1R* variants) that were affected with melanoma and their unaffected relatives.

‡ The odds ratios and 95% confidence intervals are measures of association between melanoma risk and *MC1R* variants. These odds ratios were estimated by the generalized estimating equations method using a logit link function and an exchangeable correlation matrix to take into account the correlations among the family members' melanoma affection status (affected, unaffected). The odds ratios shown in this table are adjusted for age, sex, and geographic locales.

§ *P* values for the two-sided generalized score test of association between melanoma risk and *MC1R* variants.

‖ *P* values for the two-sided generalized score test of homogeneity of the association of *MC1R* variants with melanoma risk among different geographic locales.

¶ *P* values for the two-sided trend test which tests for a change in melanoma risk with a linear increase in the number of *MC1R* variants (0, 1, ≥2).

Nonsynonymous *MC1R* variants that were not RHC variants.

** RHC variants included R151C, R160W, D294H, and D84E.

Table 4. Association of melanocortin-1 receptor (*MC1R*) variants with melanoma risk in all cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutation carriers according to the type of *CDKN2A* mutations*

	***CDKN2A* mutations that altered p16INK4a protein only**			***CDKN2A* mutations that altered both p16INK4a and p14ARF proteins**			
***MC1R* variants**	**No. of participants, affected/unaffected†**	**OR (95% CI)‡**	***P*§**	**No. of participants, affected/unaffected†**	**OR (95% CI)‡**	***P*§**	$P_{homogeneity}$‖
Individual *MC1R* variants							
V60L	82/59	5.98 (2.56 to 13.97)	.0005	103/114	2.70 (1.62 to 4.49)	.0007	.11
V92M	53/55	4.38 (1.95 to 9.84)	.0006	85/111	2.18 (1.14 to 4.17)	.03	.19
R151C	80/53	10.41 (4.59 to 23.57)	7.04×10^{-5}	122/105	4.04 (1.90 to 8.59)	.0002	.09
R160W	45/50	4.12 (1.71 to 9.95)	.002	100/101	4.40 (2.14 to 9.04)	.004	.91
No. of *MC1R* variants	195/121			263/214			
1 variant		3.63 (1.63 to 7.64)	.003		1.91 (1.09 to 3.33)	.03	.15
≥2 variants		8.38 (3.82 to 18.36)	2.15×10^{-5}		5.85 (3.09 to 11.07)	.0001	.37

* The association of each *MC1R* variable (individual *MC1R* variants, number of *MC1R* variants) with melanoma risk was estimated in *CDKN2A* mutation carriers according to whether *CDKN2A* mutation affected p16INK4a protein alone or both p16INK4a and p14ARF proteins. Analysis of each *MC1R* variable used homozygosity for the *MC1R* consensus sequence as the reference category. CI = confidence interval; OR = odds ratio.

† The number of GenoMEL participants contributing to the analysis of a given *MC1R* variable (individual *MC1R* variants, number of *MC1R* variants) that were affected with melanoma and their unaffected relatives.

‡ The odds ratios and 95% confidence intervals were estimated by the generalized estimating equations method. The odds ratios were adjusted for age, sex, and geographic locales.

§ *P* values for the two-sided generalized score test of association between melanoma risk and *MC1R* variants

‖ *P* values for the two-sided Cochran *Q* test of homogeneity of the association of *MC1R* variants with melanoma risk according to the type of *CDKN2A* mutations.

associated with red or blond hair color ($P \leq .016$) and the R151C ($P = .002$), V60L ($P = .03$), and V92M ($P = .03$) variants were statistically significantly associated with sunburn, but no variant was associated with high numbers of nevi (data not shown). Table 5 shows that when each host phenotype was analyzed separately, hair color, sunburn, and high numbers of nevi were statistically significantly associated with increase in melanoma risk, while adjusting for age, sex, and geographic locale (for red or blond hair color: OR = 3.30, 95% CI = 1.98 to 5.52; for usually or always burns: OR = 2.10, 95% CI = 1.39 to 3.17; and for high numbers of nevi: OR = 3.05, 95% CI = 1.95 to 4.78). In the presence of host phenotypes, analyzed individually or jointly, the increase in melanoma risk with any one of the four frequent *MC1R* variants remained statistically significant (Table 5). Hair color showed an additional association with melanoma risk with most variants ($.01 \leq P \leq .07$), whereas sunburn was only marginally statistically significant ($P \geq .03$) (Table 5). Moreover, an increase in the number of nevi contributed independently to melanoma risk ($.005 \leq P \leq .02$). We obtained similar results when the number of variants was analyzed. A statistically significant increase in melanoma risk with number of variants in the presence of each host phenotype or with all phenotypes was observed (for one variant, $.01 \leq P \leq .02$; and for at least two variants, $1.6 \times 10^{-5} \leq P \leq .0001$). Hair color and number of nevi, both separately and together, showed statistically significant associations with melanoma risk in addition to the number of *MC1R* variants (for hair color, $P \leq .006$; and for nevi, $P \leq 2.6 \times 10^{-5}$), whereas the association with sunburn was no longer statistically significant ($P \geq .11$). Because *MC1R* variants are much more strongly associated with red hair than with blond hair in the general population (36,37), analyses were repeated after excluding the 57 *CDKN2A* mutation carriers with red hair and showed associations between *MC1R* variants and hair color with melanoma risk similar to those shown in Table 5, indicating that blond hair was the major determinant of the odds ratios that were previously obtained when subjects with red hair were included in the analysis (data not shown). Further stratified analysis based on hair color showed that the statistically significant increase in melanoma risk that was associated with individual *MC1R* variants or number of variants was limited to subjects with brown or black hair (Supplementary Table 4, available online), thus confirming the role of *MC1R* beyond that due to pigmentation.

Association of *MC1R* Variants With Age at Diagnosis of Melanoma

Finally, to assess whether *MC1R* variants have an influence on age at diagnosis of melanoma, we examined the median ages at diagnosis of melanoma according to various categories of *MC1R* variables (individual variants, presence of any variant, number of variants, and number and types of variants). As shown in Table 6, there was a slight decrease in age at diagnosis in *CDKN2A* mutation carriers with presence of R151C or R160W variants or with increase in number and types of variants. This decrease in age at diagnosis reached marginally statistical significance in *CDKN2A* mutation carriers from Europe ($.02 \leq P \leq .05$), or when the *CDKN2A* mutation carriers were pooled across all continents ($.008 \leq P \leq .06$).

Discussion

This study investigated the associations of *MC1R* variants with melanoma risk in 815 *CDKN2A* mutation carriers from 186 families that participated in 15 GenoMEL centers across Europe, North America, and Australia. The included families had at least two cutaneous melanoma patients and the presence of a germline *CDKN2A* mutation in the family. To our knowledge, it represents

Table 5. Association of host phenotypes and melanocortin-1 receptor (*MC1R*) variants with melanoma risk in cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutation carriers from all continents*

Association of host phenotypes alone			Joint associations of host phenotypes and individual *MC1R* variants								Joint associations of host phenotypes and number of *MC1R* variants		
				MC1R variant									
				V60L (112, 99)†		V92M (80, 97)†		R151C (115, 85)†		R160W (96, 84)†			
Host phenotypes (272, 192)†	OR (95% CI)‡	*P*§	Host phenotypes and individual *MC1R* variants	OR (95% CI)‡	*P*§	OR (95% CI)‡	*P*§	OR (95% CI)‡	*P*§	OR (95% CI)‡	*P*§	Host phenotypes and No. of *MC1R* variants (272, 192)†	OR (95% CI)‡	*P*§
Hair color	3.30 (1.98 to 5.52)	.0002	Hair color	2.87 (1.10 to 7.50)	.07	3.41 (1.19 to 9.77)	.04	3.26 (1.4 to 7.6)	.01	3.03 (1.34 to 6.87)	.02	Hair color	2.48 (1.46 to 4.20)	.004
			≥1 individual *MC1*R variant	2.89 (1.56 to 5.36)	.002	2.86 (1.38 to 5.95)	.008	3.69 (1.68 to 8.12)	.002	3.18 (1.5 to 6.74)	.01	1 *MC1R* variant	1.99 (1.20 to 3.29)	.01
												≥2 *MC1R* variants	4.74 (2.68 to 8.37)	1.6×10^{-5}
Sunburn	2.10 (1.39 to 3.17)	.001	Sunburn	1.60 (.79 to 3.26)	.20	2.49 (1.05 to 5.89)	.04	2.03 (.86 to 4.82)	.10	2.49 (1.15 to 5.41)	.03	Sunburn	1.49 (.93 to 2.40)	.11
			≥1 individual *MC1R* variant	2.95 (1.55 to 5.62)	.003	2.54 (1.24 to 5.22)	.01	4.06 (1.87 to 8.85)	.001	3.67 (1.7 to 7.89)	.008	1 *MC1R* variant	1.99 (1.19 to 3.33)	.01
												≥2 *MC1R* variants	5.0 (2.76 to 9.07)	3.0×10^{-5}
Nevi	3.05 (1.95 to 4.78)	1.3×10^{-5}	Nevi	3.21 (1.49 to 6.91)	.005	2.82 (1.34 to 5.93)	.01	2.25 (1.2 to 4.22)	.02	2.14 (1.1 to 4.17)	.02	Nevi	3.0 (1.88 to 4.79)	2.6×10^{-5}
			≥1 individual *MC1R* variant	2.88 (1.47 to 5.65)	.004	2.85 (1.3 to 6.25)	.01	4.88 (2.25 to 10.6)	.0003	4.28 (2.12 to 8.66)	.002	1 *MC1R* variant	2.05 (1.16 to 3.62)	.02
												≥2 *MC1R* variants	5.69 (3.08 to 10.50)	2.1×10^{-5}
Hair color	2.34 (1.44 to 3.79)	.003	Hair color	3.05 (1.13 to 8.19)	.06	3.67 (1.28 to 10.56)	.04	3.27 (1.26 to 8.47)	.03	3.07 (1.25 to 7.53)	.03	Hair color	2.59 (1.44 to 4.64)	.006
Sunburn	2.07 (1.39 to 3.08)	.001	Sunburn	1.36 (.66 to 2.81)	.38	2.29 (.92 to 5.67)	.06	1.66 (.68 to 4.04)	.27	2.47 (1.12 to 5.43)	.03	Sunburn	1.38 (.86 to 2.21)	.19
Nevi	2.87 (1.91 to 4.31)	5.3×10^{-6}	Nevi	3.25 (1.55 to 6.82)	.004	3.25 (1.61 to 6.56)	.003	2.29 (1.21 to 4.36)	.02	2.38 (1.18 to 4.81)	.01	Nevi	3.14 (2.00 to 4.91)	6.9×10^{-6}
			≥1 individual *MC1R* variant	2.48 (1.26 to 4.88)	.01	2.44 (1.09 to 5.49)	.04	3.03 (1.4 to 6.58)	.007	2.66 (1.23 to 5.78)	.04	1 *MC1R* variant	1.98 (1.15 to 3.41)	.02
												≥2 *MC1R* variants	4.23 (2.27 to 7.87)	.0001

* The associations of host phenotypes with melanoma risk were estimated by using the following dichotomous categories: hair color (blond or red vs dark or brown), propensity to sunburn (usually or always burns vs sometimes or never burns) and nevus count (some or many nevi vs none or few nevi). The association of each *MC1R* variable (individual *MC1R* variants, number of *MC1R* variants) with melanoma risk was estimated by using homozygosity for the *MC1R* consensus sequence as the reference category. CI = confidence interval; OR = odds ratio.

† The number of affected (melanoma patients) and unaffected relatives contributing to a given analysis (host phenotypes alone, host phenotypes and individual *MC1R* variants, and host phenotypes and number of *MC1R* variants) are shown in parentheses; only the subjects who have all their host phenotypes known have been included in these analyses.

‡ The odds ratios and 95% confidence intervals were estimated by the generalized estimating equations method. The odds ratios were adjusted for age, sex, and geographic locales.

§ *P* values for the two-sided generalized score test of association between melanoma risk and any predictor variable (host phenotypes and/or *MC1R* variants).

Table 6. Association of melanocortin-1 receptor (*MC1R*) variants with median ages at diagnosis of melanoma in case patients carrying cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutations*

	Europe			North America			Australia			All continents		
***MC1R* variant**	**No. of melanoma patients**	**Median age at diagnosis**	***P*†**	**No. of melanoma patients**	**Median age at diagnosis**	***P*†**	**No. of melanoma patients**	**Median age at diagnosis**	***P*†**	**No. of melanoma patients**	**Median age at diagnosis**	***P*†**
Individual *MC1R* variants												
V60L	108			50			32			190		
0		39			31.5			33			36.5	
≥1		37	.31		34.5	.57		37	.68		36	.61
V92M	91			28			24			143		
0		39			31.5			33			36.5	
≥1		39	.17		30.5	.50		33	.43		34	.08
R151C	115			35			57			207		
0		39			31.5			33			36.5	
≥1		35	.03		29	.43		34	.92		34	.05
R160W	94			35			23			152		
0		39			31.5			33			36.5	
≥1		34	.05		31	.73		33	.90		35	.06
Any *MC1R* variant	268			115			87			470		
0		39			31.5			33			36.5	
≥1		35	.11		31	.93		34	.94		35	.13
No. of *MC1R* variants	268			115			87			470		
0		39			31.5			33			36.5	
1		36			34			35			35	
≥2		35	.10		29.5	.14		33	.85		34	.06
Types of *MC1R* variants	268			115			87			470		
0		39			31.5			33			36.5	
1 NRHC‡		38			34.5			36			37	
1 RHC§		34			31			34			34	
≥ 2 NRHC		40			31			31.5			36	
1 RHC, 1 NRHC		35			29			34			33.5	
≥2 RHC		35	.02		27	.07		33.5	.92		34	.008

* The median ages at diagnosis of melanoma were estimated in melanoma patients carrying *CDKN2A* mutations from each continent and from all three continents for each category of *MC1R* variables (individual *MC1R* variants, any *MC1R* variant, number of *MC1R* variants, types of *MC1R* variants). NRHC = nonred hair color; RHC = red hair color.

† *P* values for the two-sided Jonckeere–Terpstra test used to test for a change in age at diagnosis of melanoma with presence of individual *MC1R* variants, presence of any variant or as the number and number and types of *MC1R* variants increased.

‡ Nonsynonymous *MC1R* variants that were not RHC variants.

§ Red hair color variants included R151C, R160W, D294H, and D84E.

by far the largest analysis of *CDKN2A* mutations carriers to date that explored the associations of *MC1R* variants with melanoma risk alone, and jointly, with host phenotypes. Our study showed that both RHC and NRHC variants were statistically significantly associated with melanoma risk in *CDKN2A* mutation carriers across all three continents, strengthening the fact that the melanoma risk is not restricted to red hair color variants The joint analysis of *MC1R* variants and host phenotypes (hair color, propensity to sunburn, number of nevi), showed that, in *CDKN2A* mutation carriers, *MC1R* variants have an association with melanoma development beyond that due to pigmentation. We also found that, in addition to *MC1R* variants, both hair color and high numbers of nevi were associated with melanoma risk.

A total of 23 nonsynonymous *MC1R* variants were detected in affected and unaffected *CDKN2A* mutation carriers of the 186 melanoma-prone families, and four of these variants (V60L, V92M, R151C, and R160W) showed a frequency more than 5% in these mutation carriers across all continents. Despite the ascertainment of the families through at least two melanoma patients, the frequencies of these four variants in unaffected *CDKN2A* mutation carriers were similar to those reported in control groups from the same populations (12,18).

We found that both RHC and NRHC variants were associated with statistically significantly increased melanoma risk in *CDKN2A* mutation carriers, whereas a recent meta-analysis of melanoma case–control studies reported that most *MC1R* variants, except the most frequent NRHC variants, V60L and V92M, were associated with statistically significantly increased melanoma risk (12). The meta-analysis showed some evidence of heterogeneity for the association of V60L variant with melanoma that seemed to be because of higher risk in Mediterranean populations (12). The sample sizes in the current study did not allow investigation of the association of specific variants by country; however, an increased melanoma risk associated with V60L variant was found in all three continents. The regulation of the pigmentation process by MC1R, stimulated by the α-melanocyte–stimulating hormone (α-MSH), is mediated by its ability to increase intracellular cyclic AMP levels, which triggers downstream signaling events (11). Functional studies have shown that the V60L variant has a decreased ability to stimulate cyclic AMP levels, compared with wild-type MC1R, as observed with the RHC variants (38,39). Although the V92M variant was reported to have an ability to increase the cylic AMP levels similar to that of the wild-type *MC1R* (38,39), it was found to have a reduced affinity for the MC1R ligand, α-MSH, by other studies (40,41). Moreover, the V92M variant may also be impaired in the desensitization and internalization of MC1R, two mechanisms that occur after exposure of G protein–coupled receptors, such as MC1R, to their ligands to arrest G protein signaling (39,42). Further investigation of the functional role of *MC1R* variants in presence of *CDKN2A* mutation would be worth performing to explore how the function of *MC1R* variants is altered.

The increase in melanoma risk with increase in number of *MC1R* variants confirms previous observations in *CDKN2A* mutation–positive families (14–16). We observed this increased risk across all continents, except Sweden and Italy in Europe. The small number of Swedish *CDKN2A* mutation carriers is likely to have produced the results in these subjects because a recent Swedish case–control study reported an increase in melanoma risk with an increase in the number of *MC1R* variants, and the risk was even higher in familial cases (43). Although the Italian *CDKN2A* mutation carriers in our study did not show an association between *MC1R* and melanoma risk, previous studies of unselected case patients and control subjects from different regions of Italy showed statistically significant associations between *MC1R* variants and melanoma risk (21,44,45). However, the results of our analysis in *CDKN2A* mutation carriers were also consistent with many previous case–control studies from various populations that showed an increased risk with multiple *MC1R* variants (21,43,46–49). An increase in melanoma risk with increase in the number of *MC1R* variants was also reported in a recent meta-analysis of the association between *MC1R* variants and melanoma risk in *CDKN2A* mutation carriers (50). It must be noted that the meta-analysis of published results was conducted on a much smaller sample size compared with the present analysis of raw genotypic and phenotypic data (96 vs 186 families) and did not address several points presented here, including the association of *MC1R* variants by type of *CDKN2A* mutation and the joint associations of *MC1R* variants and host phenotypes with melanoma risk.

When examining the number and types of *MC1R* variants, we observed that the increase in melanoma risk was generally higher in the presence of RHC variants. The classification of *MC1R* variants into RHC and NRHC was primarily based on the strength of association of these variants with red hair in populations of Celtic origin (17). This classification has been evolving over time and is not entirely uniform across studies investigating *MC1R* variants and the melanoma risk. We have chosen to restrict our categorization of RHC variants to the four *MC1R* variants consistently defined as RHC variants (R151C, R160W, D294H, and D84E).

The association of *MC1R* variants with melanoma risk may vary with the type of *CDKN2A* mutations. Indeed, a molecular basis for the link between *CDKN2A* and *MC1R* has been provided by in vitro studies that showed that the increased expression of p16INK4a after exposure to ultraviolet radiation is potentiated by α-MSH through its binding to *MC1R* (51). In our analysis, the associations of *MC1R* variants with melanoma risk did not differ statistically significantly depending on whether *CDKN2A* mutations altered only the p16INK4a protein or both p16INK4a and p14ARF proteins.

It is not fully resolved whether the increased melanoma risk attributed to the *MC1R* variants is distinct from their association with pigmentation characteristics. Case–control studies have indicated that part of the association between melanoma risk and *MC1R* variants remains after stratification of phenotypic features suggesting that the association of *MC1R* is not exerted entirely through pigmentation (21,46–48). However, this issue has been scarcely investigated in *CDKN2A* mutation–positive families (15,16,21). This study showed that all four frequent *MC1R* variants, V60L, V92M, R151C, and R160W, were still associated with a statistically significantly increased melanoma risk, while adjusting for hair color or sunburn. Further stratified analysis based on hair color showed that the statistically significantly increase in risk was limited to subjects with brown or black hair, in agreement with a recent case–control study (52). These results strengthen the hypothesis that

MC1R variants may have a role in carcinogenesis in addition to its influence on pigment variation. Experimental (in vitro) studies have shown that, besides its role in pigmentation, α-MSH, which binds to MC1R, is involved in anti-apoptotic DNA repair and anti-inflammatory pathways (53–56). The additional association of hair color with melanoma risk is in agreement with results from genome-wide association studies that have identified several loci influencing pigmentation phenotypes (36,37). Our study also demonstrates that having high numbers of nevi was associated with a statistically significantly increased melanoma risk in *CDKN2A* mutation carriers, independently of *MC1R* variants. A recent genome-wide association study (57), carried out by GenoMEL, in *CDKN2A*-negative melanoma case patients and control subjects, identified independent associations of three loci with melanoma risk—16q24, encompassing *MC1R*; 11q14–q21, encompassing the tyrosinase pigmentation gene (*TYR*); and 9p21, adjacent to *CDKN2A* and the methylthioadenosine phosphorylase (*MTAP*) genes (*CDKN2A/MTAP*). The *CDKN2A/MTAP* locus was concomitantly characterized as a nevus gene (58). Further investigation of the *TYR* and *CDKN2A/MTAP* loci in families segregating *CDKN2A* mutations will allow assessment of whether common variants of these genes also modify penetrance of *CDKN2A* deleterious mutations.

Our analysis may have a few limitations. A slight decrease in age at melanoma diagnosis, which only reached statistical significance in the largest sample of European *CDKN2A* mutation carriers and in mutation carriers from all three continents, was observed with the presence of RHC variants and as the number and types of variants increased. A decrease in age at melanoma diagnosis with increasing number of *MC1R* variants was previously reported to be mostly statistically significant in melanoma patients with multiple primary melanomas (15,22). Any information on the occurrence of single or multiple primary melanoma was not available for this analysis; however, we plan to investigate in the near future whether the association of *MC1R* with age at melanoma diagnosis differs in single vs multiple primary melanoma. The association of *MC1R* variants with melanoma risk was currently assessed by comparing affected and unaffected family members using an analytical method that accounts for the familial dependence and prevents inflation of the type I error rate (59). It should be noted that the odds ratios presented here are estimates of association in families similar to the sampled families and cannot be extrapolated to the general population. In all analyses, we used age at examination rather than age at diagnosis of melanoma because the host phenotypes that were examined jointly with *MC1R* variants vary over time and were measured at the time of examination. However, repeating the analyses using age at diagnosis of melanoma patients produced similar results for the association of *MC1R* variants and melanoma risk (data not shown).

In conclusion, this study shows that melanoma risk in *CDKN2A* mutation carriers is modified by multiple factors that include *MC1R* variants, pigmentation, and nevus phenotypes. Investigation of other modifying genes, such as those identified by genome-wide association studies, may help clarify the complex mechanisms leading to familial melanoma. Such studies may have important consequences for improving melanoma risk assessment in families.

Appendix

The Melanoma Genetics Consortium (GenoMEL; http://www.genomel.org) included the following participating groups:

Europe

The participants of GenoMEL in Paris, France: Florence Demenais, Hamida Mohamdi, Valérie Chaudru, Eve Corda, Patricia Jeannin, and Eve Maubec (Inserm U946 and Université Paris Diderot, Fondation Jean Dausset-CEPH, Paris, France), Marie-Françoise Avril (AP-HP, Hôpital Cochin, Service de Dermatologie, Université Paris 5, Paris, France), Brigitte Bressac-de Paillerets, Fabienne Lesueur, and Mahaut de Lichy (Département de Génétique Moléculaire, Institut de Cancérologie Gustave Roussy, Villejuif, France).

The participants of GenoMEL in Emilia-Romagna, Italy: Maria Teresa Landi (Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD), Donata Calista, Giorgio Landi, Paola Minghetti, Daniela Capirossi, Pier Alberto Bertazzi, and Fabio Arcangeli (Dermatology Unit, Maurizio Bufalini Hospital, Cesena, Italy).

The participants of GenoMEL in Genoa, Italy: Giovanna Bianhi-Scarrà, Lorenza Pastorino, Linda Battistuzzi, William Bruno, Sara Gargiulo, Paola Ghiorzo, Sara Gliori, Sabina Nasti, Paola Origone, and Paola Queirolo (Department of Oncology, Biology and Genetics, University of Genoa, Italy; Laboratory of Genetics of Rare Hereditary Cancers, San Martino Hospital, Genoa, Italy).

The participants of GenoMEL in Leiden, the Netherlands: Nelleke A. Gruis, Frans A van Nieuwpport, Wilma Bergman, Pieter van der Velden, and Leny van Mourik (Department of Dermatology, Leiden University Medical Centre, Leiden, the Netherlands).

The participants of GenoMEL in Barcelona, Spain: Paula Aguilera, Celia Badenas, Cristina Carrera, Remedios Cervera, Francisco Cuellar, Daniel Gabriel, Melinda Gonzalez, Pablo Iglesias, Josep Malvehy, Rosa Marti-Laborda, Montse Mila, Zighe Ogbah, Joan-Anton Puig Butille, and Susana Puig (Dermatology Department, Melanoma Unit, Hospital Clinic, IDIBAPS and CIBER de Enfermedades Raras, Barcelona, Spain) and other members of the Melanoma Unit: Llúcia Alós, Ana Ancero, Pedro Arguís, Antonio Campo, Teresa Castel, Carlos Conill, Jose Palou, Ramon Rull, Marcelo Sánchez, Sergi Vidal-Sicart, Antonio Vilalta, and Ramon Vilella (Dermatology Department, Melanoma Unit, Hospital Clinic, IDIBAPS and CIBER de Enfermedades Raras, Barcelona, Spain).

The participants of GenoMEL in Lund, Sweden: Håkan Olsson, Christian Ingvar, Kari Nielsen, Anna Måsbäck, Katja Harbst, Göran. Jönsson, Åke Borg (Departments of Surgery and Oncology, Lund University Hospital, Lund, Sweden).

The participants of GenoMEL in Stockholm, Sweden: Veronica Höiom, Johan Hansson, Rainer Tuominen, Diana Lindén (Department of Oncology-Pathology, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden).

The participants of GenoMEL in Glasgow, United Kingdom: Rona Mackie, Julie Lang (Departments of Medical Genetics and Public Health, University of Glasgow, United Kingdom).

The participants of GenoMEL in Leeds, United Kingdom: Julia A Newton Bishop, Paul Affleck, Jennifer H Barrett, D Timothy Bishop, Jane Harrison, Mark M Iles, Juliette Randerson-Moor, Mark Harland, John C Taylor, Linda Whittaker, Kairen Kukalizch, Susan Leake, Birute Karpavicius, Sue Haynes, Tricia Mack, May Chan, and Yvonne Taylor (Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, Cancer Research UK Clinical Centre at Leeds, St James's University Hospital, Leeds, United Kingdom).

North America

The participants of GenoMEL in Boston, United States: Hensin Tsao, Ching-Ni Jenny Njauw (Wellman Center for Photomedicine, MGH Melanoma and Pigmented Lesion Center, Massachusetts General Hospital, Boston, MA).

The participants of GenoMEL at NCI, United States: Alisa M. Goldstein, Margaret A. Tucker, Xiaohong R. Yang (Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD).

The participants of GenoMEL in Philadelphia, United States: Peter Kanetsky, David Elder,Patricia Van Belle, Michael Ming, Nandita Mitra, Althea Ruffin,

Lello Tesema, Saarene Parossian (Center for Clinical Epidemiology and Biostatistics and Department of Biostatistics and Epidemiology, University of Pennsylvania and Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA).

The participants of GenoMEL in Toronto, Canada: David Hogg, Joanne C. Y. Loo (Departments of Medicine and Medical Biophysics, University of Toronto, Toronto, ON, Canada).

Australia

The participants of GenoMEL in Brisbane, Australia: The Principal Investigators of the Queensland study of Melanoma: Environmental and Genetic Associations (Q-MEGA): Nicholas G. Martin, Grant W. Montgomery, David L. Duffy, David C. Whiteman, Stuart MacGregor, Nicholas K. Hayward (Oncogenomics Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia).

The participants of GenoMEL in Sydney, Australia: Graham J. Mann, Richard F. Kefford, Helen Schmid, Elizabeth A. Holland (Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute and Melanoma Institute Australia, Westmead, Australia).

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